Evidence for the absence of intron H of the histidine-rich glycoprotein (HRG) gene: genetic mapping and in situ localization of HRG to chromosome 3q28-q29.

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Amino acid sequence of human histidine-rich glycoprotein derived from the nucleotide sequence of its cDNA.

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A lambda gt 11 library containing cDNA inserts prepared from human liver mRNA has been screened with an affinity-purified antibody to human histidine-rich glycoprotein (HRG) and then with a restriction fragment isolated from the 5' end of the largest cDNA insert obtained by antibody screening. A number of positive clones were identified and shown to code for HRG by DNA sequence analysis. A total of 2067 nucleotides were determined by sequencing 3 overlapping cDNA clones, which included 121 nucleotides of 5'-noncoding sequence, 54 nucleotides coding for a leader sequence of 18 amino acids, 1521 nucleotides coding for the mature protein of 507 amino acids, a stop codon of TAA, and 352 nucleotides of 3'-noncoding sequence followed by a poly(A) tail of 16 nucleotides. The length of the noncoding sequence of the 3' end differed in several clones, but each contained a polyadenylylation or processing sequence of AATAAA followed by a poly(A) tail. More than half of the amino acid sequence of HRG consisted of five different types of internal repeats. Within the last 3 internal repeats (type V), there were 12 tandem repetitions of a 5 amino acid segment with a consensus sequence of Gly-His-His-Pro-His. This repeated portion, referred to as a "histidine-rich region", contained 53% histidine and showed a high degree of similarity to a histidine-rich region of high molecular weight kininogen.

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Evidence for the Absence of Intron H of the Histidine-Rich Glycoprotein (HRG) Gene: Genetic Mapping and *in Situ* Localization of HRG to Chromosome 3q28–q29

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Histidine-rich glycoprotein (HRG) belongs to the cystatin superfamily (7) and appears to be a potential risk factor for thrombosis. An increased prevalence of elevated HRG plasma levels in patients with venous thrombosis and families with thrombophilia has been reported (1). It is interesting to note that the genes of four different members of the cystatin superfamily are located on the distal section of the long arm of chromosome 3: Stefin A (STF1) on 3q21, Kininogen (KNG) on 3q26–qter, α-2-HS-glycoprotein (AHSG) on 3q27–q28, and HRG on 3q21–qter. To further investigate the evolutionary relationship between HRG and members of the cystatin super-

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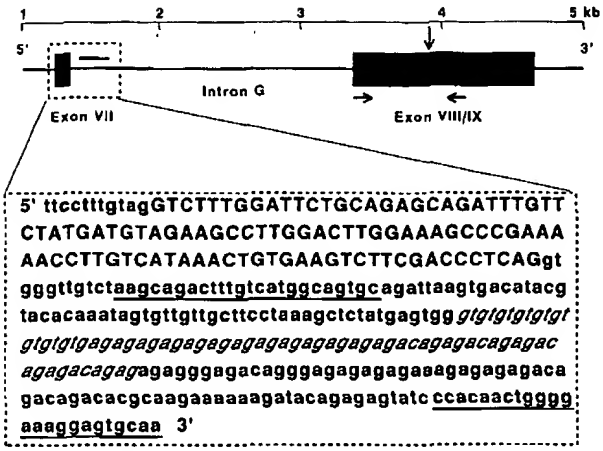

FIG. 1. Part of the HRG gene structure present in cosmid c2RBHRG-8. The intron H between exons VIII and IX proposed by Koide *et al.* (5) is indicated by a vertical arrow. Primers used to amplify the predicted intron H are indicated by horizontal arrows below exon VIII/IX. The compound repeat is indicated by a line in the dashed box. The enlargement of the dashed box shows the sequence of the repeat (GenBank Accession No. Z17218) in detail. A sequence identical to exon VII (6) is shown in capital letters and the microsatellite repeat sequence is shown in italics. Oligonucleotide primers used to amplify the repeat are underlined.

family, we isolated a cosmid that was used to refine the chromosomal localization of HRG by *in situ* hybridization. In addition, we used a dinucleotide repeat polymorphism (2) to localize HRG on the linkage map of chromosome 3q.

Using a cDNA (6) probe for HRG, a 45-kb cosmid (c2RBHRG-8) was isolated from a cosmid library constructed from DNA of a 49,XXXXY lymphoblastoid cell line. By partial sequence analysis, the presence of exons VII–IX could be confirmed. The sequences of exon VII and of the coding part of exon IX were found to be identical to the cDNA sequence reported by Koide *et al.* (6). We also found the same intron–exon boundaries for exon VII as proposed by Koide *et al.* (5). However, by sequencing the predicted boundary between exons VIII and IX, we found no intron H in this genomic clone. The absence of intron H was confirmed by PCR analysis of genomic DNA using primers chosen in exon VIII and exon IX, that amplify the predicted boundary between these exons. Genomic DNA was obtained from freshly collected blood from Dutch volunteers as described previously (9). PCR was performed in a volume of 50 μl containing 1 μg genomic DNA, 200 ng of a 5'-primer (5'-CAT GCC ACT TTT GGC ACA AAT GGG-3') in exon VIII, 200 ng of a 3'-primer (5'-TTA TTT TGG AAA TGT ATG TGT AAA AAA CAT GG-3') in exon IX, 200 μM dNTP, 1× polymerase buffer (Amersham, UK), and 0.5 unit *Taq* polymerase (Amersham). Thermocycling conditions were 1 min at 94°C (denaturation), 1 min at 55°C (annealing), and 2 min at 72°C (extension) for 30 cycles. In genomic DNA of 40 unrelated individuals, no intron was found. This finding is in contrast to the intron localization proposed by Koide *et al.* (5). They proposed an intron between the codons for amino acids 439 and 440 in the gene for HRG (Fig. 1).

Cosmid c2RBHRG-8 was used for hybridization to metaphase chromosome spreads. Labeling, hybridization, washing, and staining conditions were as described by Wijmenga *et al.* (10). Positive hybridization signals were found on the terminal region of the long arm of chromosome 3 at q28–q29. No additional spots on chromosome 3 nor on other chromosomes were seen (Fig. 2).

Clone c2RBHRG-8 was also used to identify CA-repeat regions. A highly polymorphic $(GT)_9(GA)_{13}(CAGAGA)_4$-compound repeat was found in intron G, 93 bp 3' of exon VII (Fig. 1). The repeat was used to perform linkage analysis on HRG in 40 CEPH reference families. Amplification was carried out as described previously (2). Additional marker data were obtained from the CEPH Genotype Database v6.0. Linkage was performed using the program CRI-MAP 2.4. Information from 29 markers on chromosome 3q from the CEPH database was used to insert HRG into the linkage map between the loci D3S1427 and D3S1294 (with odds for order at least 1000:1). The interlocus distances between D3S1427, HRG and D3S1294 were 13.5 and 17.8 cM in the female map and 4.7 and 2.7 cM in the male map, respectively. The D3S1262 locus, one of the Genethon markers (8), haplotypes with HRG. The observed localization of HRG by *in situ* hybridization and the calculated order on the linkage map are in good agreement with the cytogenetic localization of marker D3S1427 on 3q27 (3). The polymorphic marker in the HRG locus, mapping to the most distal band q28–q29 of chromosome 3, provides a PCR marker with a PIC of 0.80, which is useful for filling in a gap in the linkage map near the telomere.

Apart from the homology between the cystatin-like segments and the homology between the histidine-rich region of Kininogen and HRG, the evolutionary relationship between HRG and Kininogen is even more pronounced when the structures of their genes are compared. The intron localization of the two cystatin domains of HRG is very similar to the first two cystatin domains of Kininogen. Moreover, as a consequence of the absence of intron H, the entire region that is situated C-terminal to the cystatin domains of HRG is encoded by a single exon. This is comparable to the 3'-exon of the

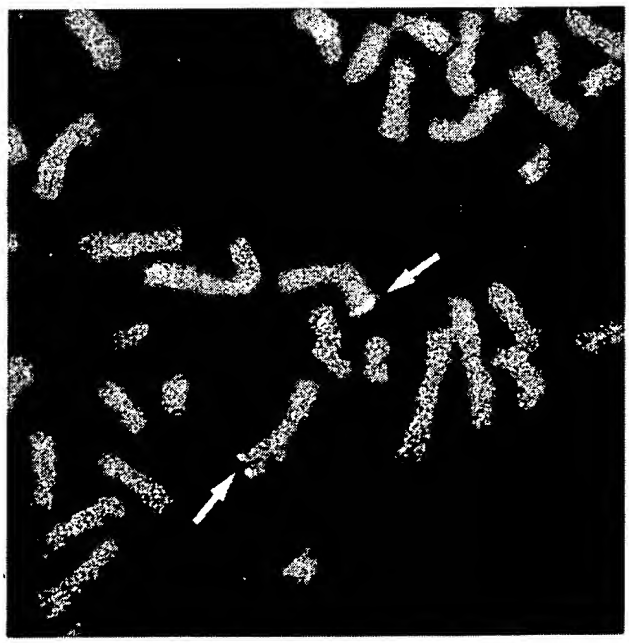

FIG. 2. *In situ* hybridization with c2RBHRG-8. Arrows indicate positive hybridization signals.

high-molecular-weight form of Kininogen (HMWK). In this splice variant of Kininogen, the region that is situated C-terminal of the cystatin domains is also encoded by one exon (4). In both HRG and HMWK, this 3′-exon represents the histidine-rich regions of the proteins. The genes of two other members of the cystatin superfamily of cysteine protease inhibitors have also been assigned to the distal part of chromosome 3q: KNG (3q26–qter) and AHSG (3q27–q28). In addition to the homologous gene structure, the physical and genetic localization of HRG close to the genes for KNG and AHSG substantiates the evolutionary relatedness of HRG to these members of the cystatin superfamily. Elucidation of the physiological function of HRG might help in understanding the homology between HRG and members of the cystatin superfamily. The availability of a PCR-based genetic polymorphism of HRG will be useful for study of the pathophysiological role of HRG in families with thrombosis.

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